

Morgan, et al.

U.S.S.N.: 09/689,343

Filed: October 12, 2000

Page 2

IN THE SPECIFICATION

At page 16, line 15-16, please amend the specification to read as follows:

sequence data. The expression of the *MseI* methylase is modulated to achieve full protection of the host genome without creating so much methylase expression as to be toxic to the host. This full methylation state is monitored by testing DNA obtained from cells in rapid logarithmic growth for protection from *MseI* endonuclease cleavage and using a construct which provides full protection under these rapid growth conditions. The *MseI* endonuclease is then expressed by amplifying the complete gene from *Micrococcus* species genomic DNA and ligating it into an expression vector designed to limit expression of the *MseI* endonuclease during cell growth prior to induction, such as pVR-24 (New England Biolabs, Inc., Beverly, Mass.). The construct is introduced into a host with appropriate genetic composition to provide sufficient regulatory capacity (U.S. ~~Application Serial No. _____~~) Application Serial No. 09/689,359 which is premodified at *MseI* sites by virtue of carrying the *MseI* methylase gene expressed on the separate compatible plasmid providing full protection against *MseI* cleavage. The *MseI* endonuclease is produced by growing the host containing the *MseI* endonuclease and methylase genes, inducing with the appropriate expression conditions, harvesting the cells and purifying the *MseI* endonuclease therefrom.

At page 25, line 4, please amend the specification to read as follows:

Morgan, et al.

U.S.S.N.: 09/689,343

Filed: October 12, 2000

Page 3

exchanging the replication origin of pLT7K for that of pACYC184. Other replication origins might also be used, such as those of pSC101 (Stoker, et al., *Gene* 18:335-341 (1982)), pSYX20 (U.S. Patent No. ~~5,262,313~~ 5,262,318), F (Shizuya, et al., *Proc. Natl. Acad. Sci. USA* 89(18):8794-8797 (1992)) or other low-copy vectors (Harayama, et al., *Mol. Gen. Genet.* 184:52-55 (1981) and Wohlfarth, et al., *J. Gen. Microbiol.* 134:433-440 (1988)). In a preferred embodiment, the vector is pVR-24.

At page 25, line 15, please amend the specification to read as follows:

In a preferred embodiment, further lowering of basal expression level is achieved by employment of a strain expressing high levels of the negative regulator of expression in the direction that allows translation of the target gene, as described in the accompanying U.S. ~~Application Serial No. _____~~
Application Serial No. 09/701,626.

At page 55, line 22, please amend the specification to read as follows:

To increase LacI repressor copy number in the host, the strain ER2833 (T7lacIq strain) was constructed as described in U.S. ~~Application Serial No. _____~~ Application Serial No. 09/689,359.

At page 59, line 20, please amend the specification to read as follows:

To enhance the stability and reproducibility of lac-based recombinant expression systems, the new host strain ER2833 (U.S.

Morgan, et al.

U.S.S.N.: 09/689,343

Filed: October 12, 2000

Page 4

~~Application Serial No. _____~~ Application Serial No.

09/689,359) was constructed, which has an copy of $lacI^q$ gene on the F' episome. Indeed, the expression stability and plasmid maintenance in the $lacI^q$ host (MseRM3) was greatly enhanced: the yield of *MseI* restriction endonuclease was $0.5-1.4 \times 10^6$ U/g wet cells.

The *MseI* restriction endonuclease purified from this strain (see Example VI) was substantially free of non-specific endonuclease and exonuclease and the final yield was

ES
conc.